I. AMENDMENTS TO THE SPECIFICATION

Please insert the following amended paragraphs as replacements for their like-numbered counterparts in the specification as filed.

[0003] The female human reproductive cycle relies on a number of gonadotropin hormones. Principle among these are the pituitary hormones follicle stimulating hormone[[,]] (FSH) and luteinizing hormone (LH). During oogenesis, the process by which the female germ cell, the ovum, is produced, occurs within a follicle. A follicle is a collection of cells in the ovary containing an oocyte (egg). Follicle maturation which ultimately leads to ovulation, is dependent on the stimulatory effects of FSH.

[0017] **In** another aspect, the invention provides a use of a PDE inhibitor for the preparation of a medicament for stimulating ovarian follicular growth in a patient. Preferably, PDE inhibitor is used in the preparation of a medicament for stimulating ovarian follicular growth in a patient undergoing ovulation induction. More preferably, the PDE is used in the preparation of a medicament for stimulating ovarian follicular growth in a patient undergoing controlled ovarian hyperstimulation for assisted reproductive technologies. The PDE inhibitor is administered starting in the stimulatory phase, before ovulation, and is preferably stopped before or on the day when the ovulatory phase is started by administration of large dose of an agent having LH-activity (such as 5,000-10,000 hCG). Most preferably administration of the PDE inhibitor stops two, one or zero days before the day on which hCG is administered. Most preferably administration of the PDE inhibitor stops on the day on which hCG is administered.

[0022] A particular aspect of the present invention provides a method of increasing the follicle maturation in an animal comprising administering to the animal a composition comprising at least one PDE inhibitor and a gonadotropin hormone, wherein the PDE and gonadotropin are administered in a collective amount effective to increase the number of human chorionic gonadotropin responsive oocytes. In particularly preferred embodiments, the PDE inhibitor is a PDE 4 inhibitor. Exemplary PDE 4 inhibitors that may be used include but are not limited to select from the group consisting of Piclamilast, Roflumilast, ~~Ariflo~~ **ARIFLO (Cilomilast)**, Filaminast, Mesopram, D4418, Arofylline, and CL1044, additional PDE 4 inhibitors may be used. Many are exemplified herein below, however it should be understood that analogs and derivatives of these compounds that have PDE 4 inhibitory activity also may be used. It is contemplated that the methods of the invention may be performed using only one PDE inhibitor. Alternatively, a cocktail of multiple PDE inhibitors may be employed. Such a cocktail may include one or more PDE 4 inhibitors in addition to one or more other PDE inhibitors. It is particularly contemplated that the composition may comprise at least one PDE 4 inhibitor and at least one other PDE inhibitor selected from the group consisting of a PDE 1 inhibitor, a PDE 5 inhibitor, a PDE 6 inhibitor, PDE 7 inhibitor, PDE 9 inhibitor, PDE 10 inhibitor, and PDE 11 inhibitor. In specific embodiments, the methods of the invention employ compositions which comprise two or more PDE 4 inhibitors.

[0042] FIG. 10A-10C. *In vitro* studies to determine the ability of PDE 4 inhibitors to induce or increase cAMP in rat granulosa cells (rat ovarian dispersate**, 10A**) and/or human FSH receptor-expressing porcine granulosa cells (JC410/FSHR**, 10B, 10C**).

[0068] Phospohdiesterases (PDE) are a family of enzymes responsible for the metabolism of the intracellular second messengers cyclic AMP (cAMP) and cyclic GMP (cGMP). PDE 4 is a cAMP specific PDE that is the major, if not sole, cAMP metabolizing enzyme found in inflammatory and immune cells, and contributes significantly to cyclic AMP metabolism in smooth muscles. PDE 4 is inhibited by the antidepressant Rolipram (4-[3-(Cyclopentyloxy)-4-methoxy-phenyl]-2-pyrrolidinone; A.G. Scientific, Inc., San Diego, CA). Rolipram was the first generation of PDE 4 inhibitors developed (see Conti, Biology of Reproduction 67:1653-1661, 2002). Subsequently, other such inhibitors have been identified, including but not limited to Piclamilast, Roflumilast, ~~Ariflo~~ **ARIFLO (Cilomilast)**, Filaminast, Mesopram, D4418, Arofylline, and CL1044. In addition, other PDE inhibitors such as Sildenafil, AS701948/1 and AS701947/1 also will be useful in the present invention. Thus, particularly preferred PDE 4 inhibitors for use in the present invention include lirimilast. (Bayer AG); CDP-840 (Celltech Group PLC), NCS-613 (Centre National de la Recherche Scientifique (CNRS) E-4021(Eisai Co Ltd), GRC-3785 (Glenmark Pharmaceuticals Ltd), IC-485 (ICOS Corp); IPL-455903 (Inflazyme Pharmaceuticals Ltd), ONO-6126 (Ono Pharmaceutical Co Ltd), Tofimilast (Pfizer Inc.), Piclamilast (Rhone-Poulenc SA (Aventis SA)), Cilomilast. (SmithKline Beecham PLC), Filaminast. (Wyeth-Ayerst Pharmaceuticals Inc), WAY-126120 (Wyeth-Ayerst Pharmaceuticals Inc0), Mesopram (Schering), and Roflumilast (Altana).

[0087] Particularly preferred PDE4 inhibitors that may be used herein include but are not limited to Roflumilast (methods and compositions for making this compound may be found in WO9501338), Piclamilast (methods of making the same are described in *J. Med. Chem.* 37:1696-1703 (1994)), ~~Ariflo/Cilomilast~~ **ARIFLO (Cilomilast)** (methods of

making the same are described in *J. Med. Chem.* 41:821-835 (1998)), Mesopram (methods of making the same are WO97/15561), Filaminast (methods of making the same are described in EP0470 805 B1).

[0133] ***Chemicals:*** Human recombinant follicle stimulating hormone (r-hFSH) and human recombinant chorionic gonadotrophin (r-hCG) were supplied by Laboratoires Serono Aubonne (LSA, Aubonne, Switzerland). Test compounds were either synthesized based on published compound synthetic methods or purchased from commercial sources. In particular, those of skill are referred to WO9501338 which teaches methods of making Roflumilast, J. Med. Chem. 1994, 37,1696-1703 for a detailed description of methods of making Piclamilast, J. Med. Chem. 1998, 41,821-835 for a description of methods for making ~~Ariflo/Cilomilast~~ **ARIFLO (Cilomilast)**, WO97/15561 for methods of making Mesopram, and EP0470 805 B1 for methods of making Filaminast. Such methods may be modified for producing other PDE inhibitors. Other test compounds (Dipyridamole, Zaprinast, Sildenafil) were either synthesized based on published compound synthesis methods or purchased from commercial sources. In addition, U.S. Patent Application No. 60/470,434 titled "Inhibitors of PDE Enzymes in Infertility," U.S. Patent Publication No. 20020103106, and PCT/EP01/14730 are incorporated herein by reference as teaching other related such compounds that may be used herein.

[0138] At ~~10h00~~ **10:00** of the morning following r-hCG administration, rats were euthanized by CO_2 asphyxia. The animals were laid on their backs and undersides were sprayed with ethanol to both sterilize and keep the hair from falling out in the dissection of the animals. With the aid of scissors and forceps the skin and muscle were cut starting

from the pubic symphisis with aboral-oral direction up to the sternum. The internal organs were exposed and the intestine was moved to one side. The ovaries, the uterine horns and the uterus body were removed clipping away the fat and the connective tissue. The entire reproductive tract was then placed into a well in a 24 well plate containing PBS (1 animal/well).

TABLE 1. FOLLICULAR GROWTH ACTIVITY OF VARIOUS PDE INHIBITORS

Compound	**PDE selectivity**	**Follicular growth activity with Low FSH?**
Papavarine	Non-selective	NO
Sildenafil	PDE1, 5, 6	YES
~~Ariflo~~ **ARIFLO (Cilomilast)**	PDE4	Inhibits follicular growth
Dipyridamole	PDE5, 6, 7, 8, 10, 11	YES
Zaprinast	PDE1, 5, 6, 7, 9, 10, 11	YES
CDP840	PDE4	Inhibits follicular growth
Tadalafil	PDE5 and 6	YES
Compound no. 31	PDE1	YES
Compound no. 33	PDE1	YES

[160] *In vivo*, two exemplary PDE 4 inhibitors, Piclamilast and Roflumilast, increased FSH-induced follicle maturation. Low concentrations of Piclamilast (0.08 and 0.4 mg/kg) were ~~was~~ not sufficient to induce follicle maturation in the absence of a low level

of exogenous FSH (FIG. 13). However, at the higher concentration of 2 mg/kg Piclamilast did induce follicle maturation (see FIG. 13). When Piclamilast is administered in the presence of low doses of FSH there was a very marked induction in follicle maturation as evidenced by the increase in number of ovulatable oocytes (FIG. 14).